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DISTRIBUTION AND FUNCTIONS OF ELASTIC FIBERS IN THE INVERTEBRATES

HUGH Y. ELDER

Institute of Physiology, University of Glasgow, Glasgow G12 8QQ Scotland, and The Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Histochemical and functional anatomical evidence has been presented in Elder and Owen (1967) and in Elder (1973) for the existence of both collagen fibers and elastic fibers in the polychaete *Polyphysia crassa* and in several other invertebrates. In view of the unique fibrous architecture of the body wall connective tissues of *Polyphysia* and of the unusual locomotory mechanism employed in that species, and upon which most of the work cited above has been based, it was of relevance to discover how widespread are the elastic fibers amongst the invertebrates. While evidence from x-ray diffraction, physical properties, biochemical analyses and electron microscopy has established the existence of collagen fibers in all metazoan groups examined, both vertebrate and invertebrate (Marks, Bear and Blake, 1949; Piez and Gross, 1959), no correspondingly unequivocal demonstration of elastic fibers has been given amongst the invertebrates and even amongst mammalian tissues elastic fibers are extremely difficult to define biochemically (Piez, 1968; Partridge, 1970; Bodley and Wood, 1972). Moreover, both histochemically and ultrastructurally there are clear differences between the invertebrate elastic fibers and those of vertebrates (Elder and Owen, 1967 and Elder, 1972). The protein "elastin," characteristic of vertebrates (Ross and Bornstein, 1969) appears to be absent from invertebrates (Piez, 1968). The only definitive test for an invertebrate elastic fiber would be the physical demonstration of the "property of rubber-like extension and elastic snap on recoil" characteristic of vertebrate elastin (Partridge, 1970, page 593); preliminary tests on the radial fibers in *Polyphysia* body wall were reported in Elder and Owen (1967) and the physical extensibility has been shown from histological sections of the body wall fixed in different configurations (Elder, in preparation).

Staining techniques, such as the spirit blue (Owen, 1959; Elder and Owen, 1967) and aldehyde fuchsin (Gomori, 1950; Gabe, 1953) methods, readily distinguish invertebrate collagenous fibers, which do not take these dyes, from other fibers which stain readily. Amongst the latter, elastic fibers may be found. As a first step, however, it was considered essential to determine how widely distributed amongst the invertebrates the spirit blue positive fibers were and in what anatomical locations they occurred.

Materials and Methods

Most of the specimens were collected in the Woods Hole region, Massachusetts. Other specimens were collected in the Cobscook Bay area of Northern Maine or were supplied by the Scottish Marine Biological Association, Millport, Isle of

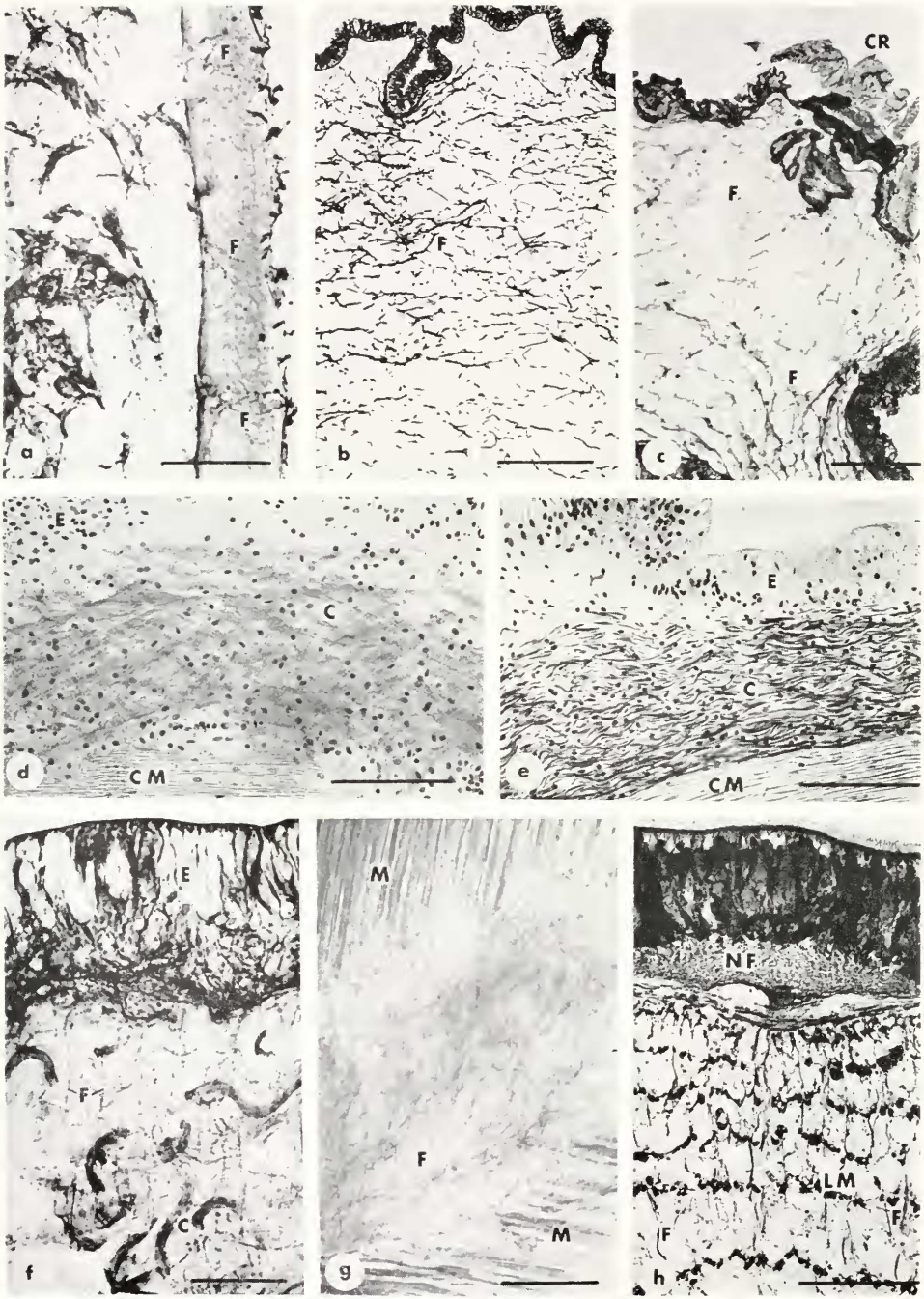

FIGURE 1. (a) Micrograph of the mesoglea of the hydroid polyp *Corymorpha pendula*, stained with spirit blue and van Gieson. The mesogleal matrix stains as for collagen but a

Cumbrae. Much of the histology was done in the Marine Biological Laboratory, Woods Hole, Massachusetts. Specimens were fixed in Bouin's fluid and embedded in paraffin or ester wax (B.D.H. Ltd.) and cut at 2–8 μm. The routine stains used were Masson's trichrome, aldehyde fuchsin (Gabe, 1953) and fast green, and spirit blue and van Gieson (Elder and Owen, 1967).

For electron microscopy specimens were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3 with 3% sucrose, post fixed in 1% osmium tetroxide in the same buffer and embedded in araldite. Sections were cut on L.K.B. I or III ultramicrotomes, stained in uranyl acetate and lead citrate and examined on A.E.I. EM6 and EM6B electron microscopes of the Departments of Zoology and Physiology of the University of Glasgow. Light micrographs were taken on a Leitz Orthoplan.

Observations on the burrowing behavior of animals were carried out at the MBL, Woods Hole. Animals were observed while burrowing into the surface of the substratum in open dishes or down the sides of a glass tank. Electronic flash photographs were made for examination and analysis.

Results

Some forty selected species from twelve invertebrate Phyla were examined histologically for the presence of spirit blue positive fibers. A detailed description of all the results is not appropriate to the present work but the major findings are summarized in Table I and preliminary accounts have been given in Elder (1966a

number of radially oriented, spirit blue positive fibers (F) are seen traversing the thickness of the mesoglea; 50 μm bar. (b) The field shows part of the exumbrellar region of the bell of the scyphozoan *Dactylometra quinquecirrha*. The epithelium is folded due to the significant shrinkage during processing. The extensive network of spirit blue positive fibers (F), organized approximately tangentially to the exumbrellar surface, is apparent; spirit blue and van Gieson stained; 200 μm bar. (c) A sector of the collenchyme and epidermis of the ctenophore *Mnemiopsis leidyi* is shown; spirit blue and van Gieson stained. Again considerable distortion has occurred during preparation but the network of spirit blue positive fibers (F) is apparent throughout the collenchyme; CR, ctene row; 200 μm bar. (d) Tangential section through the body wall of the holothurian *Leptosynapta tennis;* Masson trichrome stain. The long axis of the body is from top to bottom of the micrograph and it can be seen that collagen (C) fibers occur predominantly in right and left handed helices along the long axis of the body; CM, circular muscles; E, epidermis; 100 μm bar. (e) Transverse section through the body wall of *Leptosynapta tennis;* Masson trichrome stain. The radial integration of the thick dermal collagen layer, achieved by the wavy course adopted by the individual collagen fibers, is apparent. Immediately beneath the epidermis and above the collagen is a pale layer which contains the ossicles and does not stain with this method. Lettering is as in previous figures; 100 μm bar. (f) Transverse section through the outer layer of the dermis in *Leptosynapta;* stained with spirit blue and van Gieson. This layer, unstained in 8e above, stains intensely by this method. A number of stout, wavy collagen fibers link the epidermis to the underlying dense collagen layer (not in the field). A number of radially oriented, spirit blue positive fibers are seen traversing the deeply stained matrix of this outer dermal layer. Lettering is as above; 50 μm bar. (g) Tangential section through the gizzard of the polychaete *Aphrodite aculeata;* spirit blue and van Gieson stained. In the connective tissue between muscle layers an extensive network of spirit blue positive fibers (F) is apparent; M, muscle; 50 μm bar. (h) Transverse section through the proboscis of the hemichordate *Saccoglossus kowalewskii;* spirit blue and van Gieson stained. Densely stained spirit blue positive fibers are seen radially oriented, running within a meshwork of fine collagen fibers and linking the concentric layers of longitudinal muscle fibers, (LM); NF, nerve fiber layer underlying the ciliated epidermis; 100 μm bar.

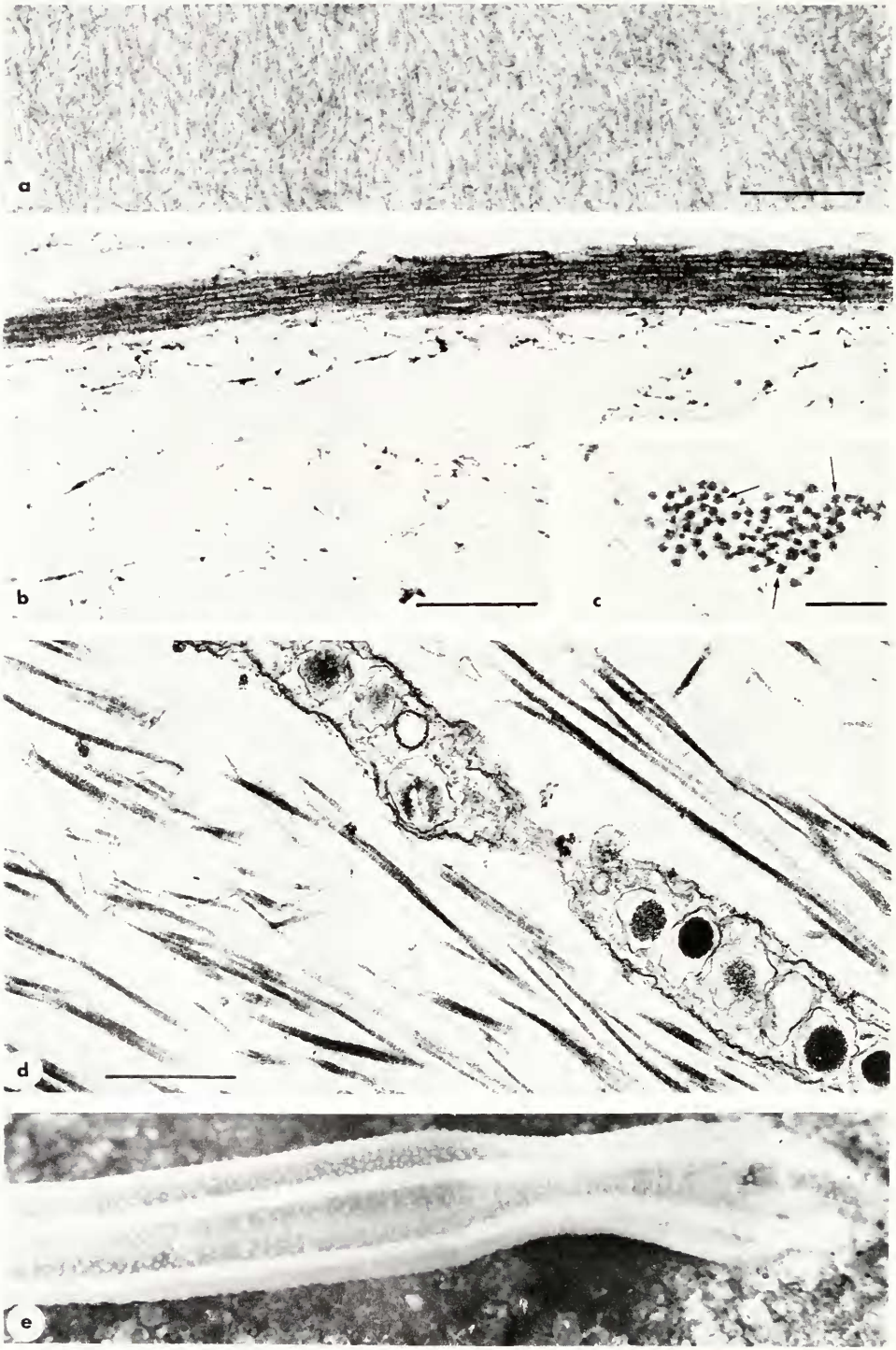

FIGURE 2.

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ÉTUDES EMBRYOLOGIQUES DANS LE GENRE *CYNORKIS (ORCHIDACEAE)*

par Yvonne VEYRET

RÉSUMÉ : Les *C. ridleyi* Dur. et Schinz., *C. ampullacea* H. Perr. et l'hybride présumé *C. lilacina* × *C. ridleyi* H. Perr. sont apomictiques autonomes absolus. L'archespore est la CMM; elle avorte ou dépasse rarement le stade dyade suivant les espèces. Ses produits ne sont pas fonctionnels. L'embryonie est soit nucellaire, soit omnisaccale diploïde; ce dernier mode est nouveau.

SUMMARY : *C. ridleyi* Dur. et Schinz., *C. ampullacea* H. Perr. and the presumed hybrid *C. lilacina* × *C. ridleyi* H. Perr. are obligate autonomous apomicts. Archespore is the MMC; generaly is aborts or give rise to dyad cells according to the species. Its products are not functionnal. Embryony is nucellar or omnisaccal diploid; this last modality is new.

Le genre *Cynorkis* groupe actuellement environ 140 espèces; l'une d'elles est asiatique, un petit nombre se rencontrent en Afrique tropicale et australe, les autres sont spéciales à la région malgache : Madagascar, Comores, Mascareignes, Seychelles; mais la majorité sont endémiques de la Grande Ile.

Ces espèces sont réparties dans 6 sections suivant le nombre de viscidies et diverses caractéristiques du rostellum. Les espèces présentement étudiées appartiennent toutes à la première section du genre, qui rassemble plus de la moitié des *Cynorkis*, mais elles y sont classées dans des groupes différents : le *C. ridleyi* Dur. et Schinz. dans le premier, le *C. ampullacea* H. Perr. dans le troisième, l'hybride présumé *C. lilacina* × *C. ridleyi* a le premier de ses parents dans le deuxième groupe.

Chez toutes ces espèces le mode de reproduction asexué était évident avant d'en avoir la preuve microscopique; en effet le pollen peut être avorté ou les pollinies rester en place et le stigmate ne porter aucune trace de pollen; malgré cela elles développent des ovules pourvus d'embryons. Dans tous les cas l'apomixie est autonome et absolue mais les modalités de la reproduction apomictique présentent des différences suivant ces espèces et en conséquence nous examinerons celles-ci successivement.

ance is quite distinct from that of collagenous fibers. In their staining properties under the light microscope and in their electron microscopical appearance these fibers resemble those described by Bouillon and Vandermeerssche (1956), though only low power micrographs were presented by these authors. The ultrastructure of the medusan fibers differs from that of the spirit blue fibers described from *Polyphysia*, *Peripatus* and molluscan tissues (Elder and Owen, 1967) in being composed of aggregates of filaments associated together apparently by cross bridges (Elder, 1966b).

A loose network of fine fibers, some 10 nm in diameter, is also present throughout the mesoglea (Fig. 2b). The filaments resemble those found in the mesoglea of *Corymorpha* above. Though not so dense as in the latter species, these filaments are probably responsible for the diffuse collagen-like staining reactions of the medusan mesogleal matrix. In the stauromedusan *Craterolophus convolutus* they form a coarse network visible with the light microscope (Smith and Elder, 1967).

In the Phylum Ctenophora the collenchyme was found to contain a network of spirit blue staining fibers (Fig. 1c) in *Mnemiopsis leidyi*, the only species examined.

Dermal connective tissues

In view of the important role which the elastic fibers of the body wall have in the locomotion of the polychaete *Polyphysia* (Elder, 1972; and 1973), it was considered worthwhile investigating the dermal connective tissue organization and composition in a variety of burrowing invertebrates. Radially oriented spirit blue positive fibers were encountered in the body wall connective tissues of at least some members of the Nemertea, Annelida, Sipunculida, Aschelminthes (Priapulida), Onycophora, Echinodermata (Holothuria) and Hemichordata.

(*a*). *Apodous holothurian body wall.* It was of great interest to find a thick dermal connective tissue layer comprising both collagenous and spirit blue positive fibers in the burrowing apodous holothurian *Leptosynapta tenuis*. The collagen fibers are organized in a three dimensional lattice which would allow radial, circumferential or longitudinal extension. Tangential sections (Fig. 1d) show that the fibers are organized in right and left handed helices around the long axis of the body while transverse (Fig. 1e) or longitudinal sections reveal that the fibers pass from one level to another, integrating the system radially. In the superficial levels of the dermis the collagen fibers are sparse but stout bundles are always found traversing the connective tissue to attach to the epidermal basement membrane (Fig. 1f). Most of the volume of the superficial dermal layer is occupied by a "felt-like" network of fine fibers which stain with the spirit blue and aldehyde fuchsin techniques (Fig. 1f). Radially oriented spirit blue positive fibers are also readily demonstrated traversing this layer (Fig. 1f). It is not clear whether the latter are discrete fibers or simply condensations of the felt-like network. While the spirit blue positive "felt" and the radial fibers are most easily seen in the superficial dermal layer, the radial fibers can be traced inwards through the collagen lattice to the muscle layer and the fine fiber network forms an envelope investing the collagen bundles. Under the electron microscope the fine "felt" and the collagen fibers have been found (Fig. 2d) but the radially oriented fibers have not yet been located.

Staining with alcian blue 8GX and Hale's dialyzed iron methods revealed a distribution of acid mucopolysaccharides similar to that of the fine fibrous network. While the radially oriented fibers are stained they do not appear to take these dyes more strongly than the surrounding felt-work, as they might be expected to do if they are simply condensations of that network. Fullmer (1960) has shown that aldehyde fuchsin stains some acid mucopolysaccharides after peracetic oxidation and it may be that the affinity of the fine network fibers for spirit blue or aldehyde fuchsin after permanganate oxidation is also due to the presence of acid mucopolysaccharides.

The bases of the epithelial cells are separated from each other by spaces, apparently fluid filled, which frequently extend through most of the height of the epithelium. Tangential sections through the epithelium, stained as above for glycosaminoglycans, show each cell outlined by this space, strongly stained for acid mucopolysaccharides. Electron micrographs reveal that the fine felt-work fibers penetrate radially through the space to attach apically by hemi-desmosomes.

(*b*). *Burrowing mechanism in* Leptosynapta. In an attempt to determine the significance of the complex dermal connective tissue construction, observations were made on the burrowing mechanism of *Leptosynapta*. Electronic flash photographs were taken of specimens moving over the substratum and burrowing into it and of animals burrowing in sand sandwiched between glass plates. Coelomic pressure recordings using a Statham pressure transducer and pen recorder have also been made (Hunter and Elder, 1967). These studies will be reported fully elsewhere. For present purposes it is sufficient to note that, with reference to *Leptosynapta*, previous accounts of the burrowing mechanism in holothurians (Gerould, 1897; Clark, 1901; Yamanouchi, 1926, 1929) summarized in Trueman and Ansell (1969) are inadequate or erroneous. Burrow excavation is performed by the lateral scraping of the ring of circum oral tentacles, and body progression is achieved by means of direct peristaltic waves involving simultaneous longitudinal and circular muscle contraction. At no phase of the burrowing activity is a "terminal anchor" formed and the periodic contractions of the longitudinal musculature are associated with burrow consolidation (Hunter and Elder, 1967), not with pulling the body up to a terminal anchor (Trueman and Ansell, 1969). Figure 2e shows a peristaltic constriction travelling anteriorily along the body of *Leptosynapta* during forward locomotion. This type of locomotory and burrowing mechanism has been described before only for the polychaete *Polyphysia crassa* (Elder, 1973).

During the passage of the peristaltic constriction the radial dimension of the body wall probably increases significantly. The construction of the three dimensional collagen lattice allows radial increase when circular and longitudinal muscle are contracted simultaneously and, as in *Polyphysia*, the radially oriented spirit blue fibers are probably extended in this configuration of the body wall.

(*c*). *Pedate holothurian body wall.* Sections of the pedate holothurian *Thyone briareus* revealed a thick dermal layer, the staining reactions of which suggest that it is composed principally of dense collagenous layers. A suggestion of spirit blue staining material was present but no discrete fibers were distinguishable.

(*d*). *Sipunculid burrowing and body wall structure. Golfingia gouldi* may be taken as representative of burrowers of the terminal anchorage type. Regions of the trunk form a penetration anchor during forceful protrusion of the proboscis

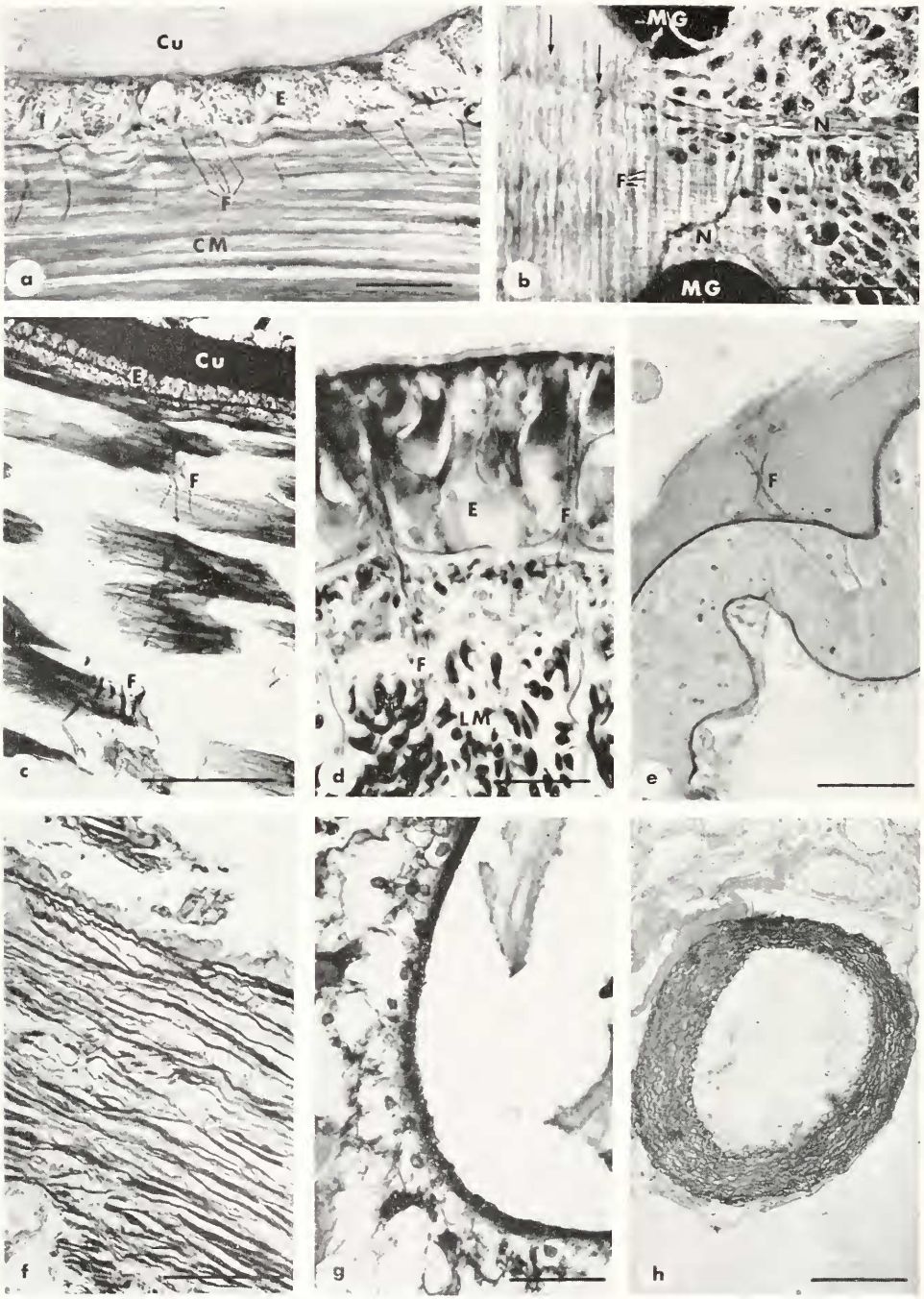

FIGURE 3. (a) A transverse section through the outer layers of the body wall of the sipunculid *Golfingia gouldi* in the trunk region; spirit blue and van Gieson stained. Part of

into the substratum. The everted proboscis then dilates to form a terminal anchor while the longitudinal muscles contract to pull the body forward (Clark, 1964; Trueman and Ansell, 1969). While coelomic pressures of only a few cm of water are employed during burrowing in *Leptosynapta,* with peaks of around 20 cm during burrow consolidation, *Golfingia* was found to utilize pressures of up to 250 cm during rapid burrowing (Hunter and Elder, 1967). A further contrast with burrowers of the lateral scraping type lies in the structure of the body wall. The trunk region of *Golfingia gouldi* possesses a very thick collagenous cuticle and a very thin dermal connective tissue layer (Hyman, 1959 and present observations, Fig. 3a). Radially oriented spirit blue positive fibers are, however, found traversing the thick circular muscle layer (Fig. 3a and Elder and Owen, 1967). At their outer extremities these fibers attach to longitudinally oriented spirit blue positive fibers of the dermal layer (Fig. 3b) and at their inner extremities to the connective tissue around the innermost circular muscle fibers. It is probable that these spirit blue positive fibers are elastic for they follow a straight course in sections from narcotised animals. However, the thickness of the circular muscle layer must increase significantly on contraction and at the same time the length of the longitudinal muscles and longitudinally oriented spirit blue fibers must also increase. The function of the radial fibers is probably to retain the position of the longitudinal muscle blocks relative to the epidermis and cuticle, irrespective of circular muscle contraction.

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the stout, lamellated cuticle (Cu), which stains like collagen, overlies the cuboidal epithelium (E). From the thin dermis radially oriented spirit blue positive fibers (F) extend through the circular muscle layer (CM); 20 μm bar. (b) Tangential section through the outer layers of the body wall of *Golfingia* in the trunk region; Aldehyde fuchsin and fast green stained. The bases of the epithelial cells with granular cytoplasm are seen to the right and the circular muscle layer to the left. The margins of mucous glands (MG) are seen top and bottom, with associated nerve fibers (N). The system of fine, longitudinally oriented, "spirit blue positive" fibers is seen running within the dermis. These longitudinal fibers link at intervals of every three or four muscle fibers with rows of radially oriented, spirit blue positive fibers (arrows). Other lettering is as above; 50 μm bar. (c) Transverse section through the outer layers of the body wall of the aschelminth *Priapulus caudatus* in the trunk region; spirit blue and van Gieson stained. Unlike the cuticle of *Golfingia* above, the stout cuticle of *Priapulus* stains deeply with spirit blue. Radially oriented, spirit blue positive fibers are seen linking the connective tissue sheaths around the circular muscle fibers. Lettering is as in the previous plates; 50 μm bar. (d) Transverse section through the outer layers of the body of the heteronemertean *Micrura leidyi;* spirit blue and van Gieson stained. Radially oriented, spirit blue positive fibers traversing the outer longitudinal muscle layer (LM) aggregate into bundles which traverse the narrow dermis and penetrate into periodic clefts amongst the ciliated epithelial cells. Lettering is as in previous plates; 20 μm bar. (e) Transverse section through the intestinal wall of the polychaete *Polyphysia crassa;* spirit blue and van Gieson stained. The gut lumen is to the bottom of the field. Spirit blue positive fibers form networks in the walls of the peri-intestinal haemal sinus and periodic columns of radially oriented fibers (F) traverse the sinus lumen; 50 μm bar. (f) Transverse section through the wall of the posterior aortic bulb in the bivalve mollusc *Mya arenaria;* spirit blue and van Gieson stained. Spirit blue positive fibers form concentric lamellae interspersed with muscle fibers and collagen. The lumen is to the top of the field; 100 μm bar. (g) Transverse section of the wall of the dorsal abdominal artery of the crustacean *Nephrops norvegicus;* spirit blue and van Gieson stained. A single, stout, spirit blue positive lamella is present, surrounded by a cuboidal epithelium; 50 μm bar. (h) Transverse section of the anterior dorsal artery in the xiphosuran *Limulus polyphemus;* stained with spirit blue and van Gieson. Concentric layers of spirit blue positive fibers are interspersed with muscle fibers; 200 μm bar.

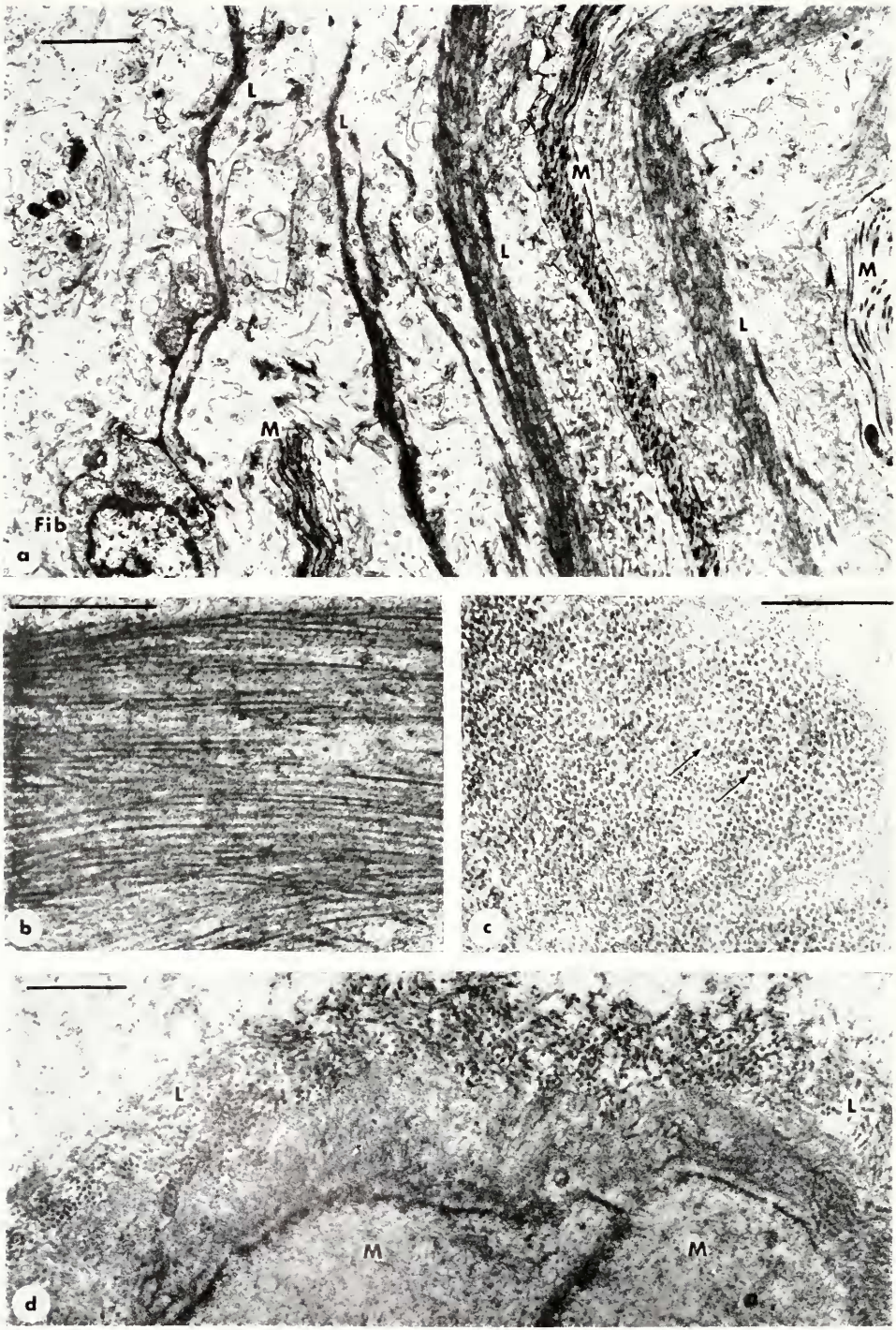

FIGURE 4.

(*e*). *Nemertean parenchymal fibers.* A similar case can be made for the properties and functions of spirit blue positive fibers which follow direct radial courses through the muscle layers of the body of *Micrura leidyi* and other nemertean worms (Fig. 3d). The fibers do not attach to the base of the epithelium but penetrate between the cells in spaces, apparently fluid filled, which periodically separate the bases of the epithelial cells (Fig. 3d), and extend at least half the height of the epithelium.

(*f*). *Connective tissues of the hemichordate proboscis.* Amongst the Hemichordata, a complex system of radially oriented spirit blue positive fibers is found associated with collagen fibers linking the nerve fiber layer of the neurectoderm and the concentrically arranged longitudinal muscle fiber layers in the proboscis of *Saccoglossus kowalewskii* (Fig. 1h).

(*g*). *Priapulid body wall.* Spirit blue positive fibers are found radially oriented amongst the circular muscle fibers of the Aschelminth *Priapulus* (Fig. 3c). Careful inspection of sections, however, suggests that these fibers are simply condensations of material which forms a sheath around each muscle fiber. Since, at least in the middle of each segment where the majority of these radially oriented condensations are found, the muscle fibers are well spaced contraction may not result in stretching of the radial elements. Unlike the cuticle of annelids and *Golfingia* which stain like collagen, the thick cuticle of *Priapulus* is strongly spirit blue positive. There is, however, no evidence to suggest that either the cuticle or the perimuscular sheaths have elastic properties. It is possible that the spirit blue affinity of these elements in *Priapulus* may, as in the case of the network of fine filaments in the dermis of *Leptosynapta,* be due to the presence of acid mucopolysaccharides.

(*h*). *Annelid body wall fibers.* Amongst the annelids the distribution of spirit blue fibers in the dermis of *Polyphysia* has been described by Elder (1972) and Owen (1959) figures spirit blue positive fibers in the body wall of *Lumbricus.* Similar fibers are present also in the Hirudinea (Elder and Owen, 1967). Several polychaete species were examined in the present series and spirit blue positive fibers were found to be of widespread occurrence in the group. Figure 1g shows a network of fibers between muscle layers in the gizzard in the errant polychaete *Aphrodite.*

Blood vessels

The blood vessels of a number of invertebrate groups were examined as a possible location in which elastic fibers might be found; spirit blue positive fibers

FIGURE 4. (a) Low power electron micrograph of part of the wall of the posterior aortic bulb in the bivalve *Mya arenaria.* The lumen lies out of the field to the right. Concentric "spirit blue positive" lamellae (L) are interspersed with muscle cells (M), collagen (C) and fibrocytes (Fib), U and Pb stained, 2 μm bar. (b) In this electron micrograph the fibrils (some 21 nm in diameter) which comprise the single lamella in the dorsal abdominal artery of *Nephrops norvegicus* are seen in longitudinal section. A faint periodicity is apparent in the densely staining fibrils; U and Pb stained; 0.5 μm bar. (c) The fibrils which comprise the lamella of the *Nephrops* artery are seen in transverse section in this electron micrograph and appear to be comprised of aggregates of finer filaments. The number of component filaments may be variable (arrows); U and Pb stained; 0.5 μm bar. (d) Part of one of the "spirit blue positive" lamellae (L) of the anterior dorsal artery of *Limulus polyphemus* is seen in this electron micrograph. As in the crustacean *Nephrops* above, the lamella is composed of electron dense fibrils which may be aggregates of finer sub units. A layer of amorphous material lies between the lamella and the periphral sarcoplasm of two obliquely sectioned muscle cells (M); 0.5 μm bar.

were found in all species examined. In the peri-intestinal haemal sinuses of the polychaete *Polyphysia* spirit blue positive fibers are located, radially oriented, traversing, the sinus between the intestinal and mesothelial surfaces (Fig. 4d). Blood pressure in this region of the circulatory system is probably neglible but it has been suggested (Elder, 1973) that movements of the body wall may play an important role in compressing the sinuses and propelling the blood. At any particular locus distension of the sinus would tend to extend the spirit blue positive fibers. Radially oriented spirit blue positive fibers were similarly found traversing the lumen of the peri-intestinal haemal sinus in *Thyone briareus*.

In the Mollusca spirit blue positive fibers were found disposed in concentric layers in the cephalic artery of the cephalopods *Loligo peali* and *Eledone cirrhosa* and in the aortic bulb of the bivalve *Mya arenaria*, (Fig. 3f). They have also been found amongst the viscera of the Pacific polyplacophoran *Cryptochiton* (Elder and Owen, 1967). Ultrastructurally the concentric lamellae of the spirit blue staining fibers in *Mya* artery (Fig. 4a) closely resemble those of the radial fibers from the dermis of *Polyphysia* (Elder, 1966b; Elder and Owen, 1967). The fine filaments of some 3 nm diameter which constitute the lamellae appear to be disposed in a predominantly circumferential orientation. Distension of these vessels would tend to extend the muscle fibers and to stress the concentric fiber layers circumferentially.

In the crustacean *Nephrops norvegicus* the structure of the dorsal abdominal artery is strikingly different from that of the molluscan vessels described above. Columnar cells lining the haemocoel surround a single stout spirit blue positive lamella which is lined by squamous endothelial cells (Fig. 3g). Electron micrographs reveal that the thick lamella is made up of fibers oriented largely along the long axis of the vessel. Moreover, the fibers appear to be compound, comprising from 3–7 filaments each some 5 nm in diameter (Figs 4b and 4c). In this respect they resemble the compound fiber type found in scyphozoan mesoglea.

The xiphosuran *Limulus polyphemus* has a more extensive vascular system than that of other arthropods. The complex structure of the anterior dorsal artery in which concentric spirit blue positive lamellae are interspersed with muscle fibers is shown in Figure 3h. Ultrastructurally these lamellae are composed of fibers longitudinally oriented, as in *Nephrops* (Elder, 1966b), and are found adjacent to extensive areas of amorphous, electron dense material initimately associated with the muscle fibers (Fig. 4d).

Discussion

The remarkable range of body form of which *Metridium* at least is capable amongst Anthozoa has been described by Batham and Pantin (1950) and a three dimensional collagen lattice was described in *Calliactis* and *Metridium* by Chapman (1953a) who also drew attention to the visco-elastic properties of the mesoglea. These properties were also studied by Alexander (1962) who described a long period reversible deformation which could not be ascribed to the known properties of the collagen rich mesoglea. Recently Gosline (1971a and 1971b) has carried out extensive tests on *Metridium* mesoglea which show that the properties of extensibility and elasticity can be accounted for by the presence of a high molecular weight amorphous polymer network in the mesogleal matrix, the collagen being

mainly a "reinforcing filler" which provides short term rigidity (Gosline, 1971b). The physical properties of *Metridium* mesoglea seem admirably adapted to the slow postural changes and very variable enterocoelic volume in this animal. Further studies may reveal instructive differences in the mesogleal structure and properties in other species with less extensible bodies, such as *Calliactis,* or more active habits, such as the burrowing anemone *Peachia* (Ansell and Trueman, 1968) or the swimming anemone, *Stomphia* (Robson, 1966). Thus although the anthozoan mesoglea displays elastic properties, the absence of any fibrous elements with such properties need not be surprising.

TABLE I

Phyletic and anatomical distribution of spirit blue positive fibers in invertebrate tissues examined

Phylum and sub phylum	Class and sub class	Species	Anatomical location of spirit blue fibers
CNIDARIA	HYDROZOA	Hydra pseudoligactis	—
		Tubularia larynx	—
		Corymorpha pendula	? polyp mesoglea
		Gonionemus murbachii	medusan mesoglea
	SCYPHOZOA	Dactylometra quinquecirrha	medusan mesoglea
		Lucernaria quadricornis	mesoglea
		Haliclystus auricula	"
		Craterolophus convolutus	"
	ANTHOZOA	Actinia equina	—
		Haloclava producta	—
CTENOPHORA	TENTACULATA	Mnemiopsis leidyi	collenchyme
PLATYHELMINTHES	TURBELLARIA	Bdelloura candida	—
		Stylochus zebra	—
	CESTODA	Schistocephalus solidus	—
NEMERTEA	ANOPLA	Micrura leidyi	radial in muscles & mesenchyme
		Cerebratulus lacteus	radial in muscles & mesenchyme
ANNELIDA	POLYCHAETA	Arenicola cristata	body wall
		Polyphysia crassa	body wall & blood vessels
		Scalibregma inflatum	body wall
		Nereis virens	body wall
		Aphrodite aculeata	gizzard
	OLIGOCHAETA	Lumbricus terrestris	body wall
	HIRUDINEA	Hirudo medicinalis	body wall
SIPUNCULIDA		Golfingia gouldi	body wall
ASCHELMINTHES	PRIAPULIDA	Priapulus caudatus	? body wall
MOLLUSCA	POLYPLACOPHORA	Cryptochiton species	visceral connective tissues
	GASTROPODA	Helix aspersa	artery
	BIVALVIA	Mya arenaria	aortic bulb
	CEPHALOPODA	Loligo pealei	cephalic artery
		Eledone cirrhosa	" "
ARTHROPODA	CRUSTACEA	Nephrops norvegicus	dorsal abdominal artery
	MEROSTOMATA	Limulus polyphemus	cephalic artery
ONYCOPHORA		Peripatoides novae-zelandiae	body wall
ECHINODERMATA	HOLOTHURIA	Leptosynapta tenuis	body wall
		Thyone briareus	intestinal haemal sinus
HEMICHODATA	ENTEROPNEUSTA	Saccoglossus kowaleswkii	proboscis

Amongst the hydrozoan polyps a system of crossed fibers parallel to and perpendicular to the oral-aboral axis in whole preparations of isolated mesoglea of *Hydra* has been described by Hausman and Burnett (1969). Their physical and histochemical study revealed the presence of elastin-like and collagen-like proteins and they concluded that the normal movements of *Hydra* could be accounted for by the presence of the elastin-like protein of the fibers. Electron micrographs revealed a fine fibrous network of 6–8 nm fibrils but, as in the present study with *Corymorpha,* the stouter fibers seen in the light micrographs were not found; Hausman and Burnett (1969) suggest that the latter are aggregations of the fine fibrils into fibers some 0.3 μm in diameter.

The divergent views of Bouillon and Vandermeerssche (1956), who suggested that the fibers of the medusan mesoglea were elastic, and those of Chapman (1953a, 1953b, 1959, 1966), who has presented convincing evidence of the presence of collagen in the mesoglea have been discussed by Elder and Owen (1967) who concluded that two distinct types of fiber were present, collagen and a type of invertebrate elastic fiber.

In previous theoretical considerations of the role of the scyphozoan mesoglea it has been assumed that the elasticity of the mesoglea serves as the antagonist for the sub-umbrellar circumferential muscles and that the radial connective tissue fibers transversing the mesoglea are stretched during contraction of the bell (Chapman, 1958, 1966). In a recent study of the swimming of medusae Gladfelter (1970) found that in many species the mesoglea does not form a mechanically homogeneous structure but has structural joints at which the bulk of bell deformation occurs. He also found that in a given medusa the amount of stretch in a region of the bell wall during contraction is inversely proportional to the number of fibers per unit area there and considers that the major role of mesogleal fibers is that of "maintaining the radial integrity of the bell during deformation." Therefore in the light of Gosline's (1971a and 1971b) work showing that the elastic properties of an anthozoan mesoglea reside in the matrix rather than in the fibrous connective tissue components, the question of whether the undoubted elasticity which the bell mesoglea possesses resides in the stout fibers or in the mesogleal matrix remains an open one.

Hyman (1940) noted the presence of a network of connective tissue fibers in the ctenophoran collenchyme and in volume V (Hyman, 1959) she supported the view of the origin of ctenophores from the trachyline hydrozoa. It is possible therefore that the spirit blue fibers observed in *Mnemiopsis* are similar to, and derived from, the type of fibers found in the hydromedusan mesoglea.

Attention has been drawn to the convergent development in *Peripatus* and *Polyphysia* of a thick collagenous dermal layer in the body wall with radially oriented elastic fibers (Elder and Owen, 1967). The extension which the latter undergo in *Polyphysia* when simultaneous circular and longitudinal muscle contraction occurs during the locomotor cycle has been estimated (Elder, in preparation). It is probably of significance therefore that, in its behavior, *Peripatus* is adapted to penetrating crevices. Manton (1961) has found that *Peripatus* can pass through a hole only one ninth the normal diameter of the body and describes how the animal passes the body through the aperture by sequential constriction of the segments. It seems probable that the elaborate dermal connective tissue will prove to have a function in freeing the underlying musculature from the restricting influence of the thick integument in a manner analagous to that in *Polyphysia*.

An even more significant convergence of dermal structure and burrowing behavior is found in the comparison of *Polyphysia* and *Leptosynapta*. In both, burrowing excavation is achieved primarily by the lateral scraping action of tentacles at the anterior end, the trunk forming a *point d'appui* which allows the head to press continuously forwards. Trunk progression is achieved by means of direct peristaltic waves with constrictions involving simultaneous circular and longitudinal muscle contraction; no terminal anchor is involved. A very flexible body wall structure appears to be a prerequisite of this type of locomtion involving the

simultaneous contraction of both muscle layers and is achieved in both animals by an elaborate three dimensional collagen lattice and a system of radially oriented fibers, probably elastic in properties. This method of burrowing, examples of which have not been described before, utilizes, and indeed would demand (Mettam, 1969), very low coelomic pressures at least in *Leptosynapta* (Hunter and Elder, 1967) and probably also in *Polyphysia* (Elder, 1973). In *Polyphysia* both the neuromuscular activity patterns and the connective tissue fiber architecture have probably evloved from sand burrowing ancestors as adaptations to burrowing in the soft mud habitat (Elder, unpublished). It is probably significant that, while most burrowing animals are closely adapted to a particular type of substratum (Yonge, 1949), *Leptosynapta tennis* (= *Synapta inhaerens* see Smith, 1964) lives and burrows in sand, muddy sand or even in pure mud (Clark, 1901). It is suggested that this degree of freedom in choice of habitat is conferred by the unique burrowing mechanism. The degree of development of the dermal connective tissues and coelomic pressures employed by these lateral scraping excavators contrasts strongly with the slight development of the dermis and relatively high coelomic pressure recorded in terminal anchorage burrowers such as *Golfingia* and *Priapulus*.

The presence of radially and longitudinally oriented spirit blue staining fibers which are most probably elastic has been noted above in the body wall of *Golfingia gouldi*. Although spirit blue staining elements are present, radially oriented in the circular muscle layer of the body wall of *Priapulus* the case for their being elastic is much weaker. In another group of the aschelminthes, the Acanthocephala, the presence of radially oriented spirit blue positive fibers in the body wall of the trunk region has been noted by Hammond (1966) although no comment was offered as to their possible function. Nicholas and Mercer (1965) have presented electron micrographs of the radially oriented fibers in the trunk wall of an acanthocephalan, which reveal a strong resemblance to the ultrastructure of the radially oriented fibers in the dermis of *Polyphysia* (Elder and Owen, 1967 and Elder, 1972).

Robson (1957) described the existence of fluid filled spaces between the bases of the epithelial elements of the musculo-epithelial cells of *Metridium*. She stated that the function of this sub-epithelial fluid compartment was to enable the epithelium to follow rapid muscular contractions without delay owing to the hydrostatic action of the fluid in thrusting it outwards. Similar fluid filled spaces have been noted between the bases of the epithelial cells of *Leptosynapta* and *Microura*. It is probably of significance that, in common with *Metridium*, these animals have highly deformable body walls. Even in *Polyphysia*, in which the deformability of the body wall is probably greater than in most other cuticularized animals, subepithelial fluid spaces of this type are absent and the epithelium and cuticle conform to body wall deformation by folding rather than by increase in thickness (Elder 1972, 1973).

The finding of a thick and predominantly collagen-staining dermis in the holothurian *Thyone* agree with the results of Piez and Gross (1959) who examined the dermal connective tissue of *Thyone* by light and electron microscopy and performed amino acid analyses. In addition to the abundant collagen fibrils of 15–200 nm diameter and 65 nm periodicity these authors also figured and noted a second, unidentified type of "very thin 'beaded' fibril" (Piez and Gross, 1959,

page 27). It remains to be shown if aggregates of the beaded fibrils are responsible for the suggestion of spirit blue staining noted above and what properties and functions this second type of fiber have.

On the basis of staining characteristics and anatomical distribution elastic fibers have been described in molluscan tissues for more than a century (Leydig, 1854). On the basis of negative result with orcein Argaud (1908, 1909) and Jullien, Cardot, Kipplinger and Claudey (1956) concluded that elastic fibers were absent from the skin of cephalopods. But using Gomori's (1950) aldehyde fuchsin Jullien, Cardot and Kipplinger (1957, 1958) have reinvestigated previous descriptions and reversed their own previous conclusion. They particularly noted the similarity between the structure of cephalopod arteries, with respect to the concentric distribution of (presumed) elastic lamellae, which they figure, and the structure of vertebrate elastic arteries. Systolic pressures of up to some 50 mm Hg have been recorded in the cephalic artery of *Octopus* during activity (Johansen and Martin, 1962). Using Weigert's resorcin and orcein, Wetekamp (1915) described elastic fibers in a variety of tissues, including the blood vascular system of the large bivalve *Anodonta cellensis*. Ventricular systolic pressures of 3 mm Hg at the normal resting level and up to almost 8 mm Hg during active pedal probing have recently been recorded from *Anodonta* (Brand, 1972). In bivalves, such as *Mya*, with well developed posterior aortic bulbs periodic pressures greater than these may occur if, as Brand (1972) suggests, blood is forced back into the bulb during siphon retraction.

Amongst the arthropods literature contains occasional reference to the identification of elastic fibers, usually on the basis of staining techniques. Thus Boissezon (1930) records elastic fibers associated with the anterior intestine of insect *Culex* and Nutting (1951) figures elastic fibers in the dorsal diaphragm of orthopterans. Mabillot (1954, 1955) reports the presence of elastic fibers in the digestive tract of the crustacean *Gammarus* and Barnes and Gonor (1958) found a loose meshwork of elastic fibers associated with the nerve cord of the cirripede *Pollicipes*. Lacombe (1970) believes that elastic fibers are involved in cement extrusion from the cement glands of some large balanid barnacles. And in the Xiphosura elastic ligaments suspending the heart and elastic fibers in the anterior pericardial wall have been described by Krumbach (1935). In the present investigation arteries in the Norway lobster *Nephrops norvegicus* and the king crab *Limulus polyphemus* were found to have spirit blue staining fibers. Systolic pressures of 9–20 mm Hg have been recorded from the posterior dorsal aorta of *Homarus americanus* (Scheer, 1963). It seems possible that the spirit blue staining lamellae have elastic properties but further studies on the circulatory dynamics of these invertebrates are required.

Amongst mammalian tissue fiber types it is clear that, on the basis of the staining properties described above and in section 1, the invertebrate elastic fibers most clearly resemble the pre-elastic and oxytalan fibers (Fullmer and Lillie, 1958; Fullmer, 1960). Argaud (1908, 1909) had previously reached a similar conclusion. He noted that the molluscan fibers had the physical characteristics of vertebrate elastic fibers but would not take elastin stains and in these respects resembled the first formed fibers in young human embryos. Ultrastructural studies have revealed further similarities between the invertebrate fibers and the oxytalan fiber

(Carmichael and Fullmer, 1966) and "elastin" precursors (Ross and Bornstein, 1969, 1970, 1971). All three fiber types are comprised of beaded filaments which stain densely with the cationic stains, uranyl acetate and lead citrate. In these respects they differ markedly from the "amorphous component" seen in ultra-thin sections of mature mammalian elastic fibers which stains with anionic stains such as phosphotungstic acid but remains unstained after application of the lead and uranium salts. There appears to be variation in the dimensions of the beaded filamentous elements of both the invertebrate fibers (Elder, 1966b) and the mammalian elastic fiber precursors (Ross and Bornstein, 1969).

The mechanism of the staining reaction between spirit blue and the invertebrate fibers remains unknown. Of a variety of stains tested (Elder and Owen, 1967) it most closely resembles aldehyde fuchsin in its staining properties. Fullmer (1965) comments that mammalian elastic fibers have a remarkable affinity for phenols, napthols and ferric salts and it is significant that all the common elastic stains (except aldehyde fuchsin) contain phenols. Aldehyde fuchsin, formed when paraldehyde reacts with basic fuchsin (Gomori, 1950) probably contains aromatic amines which may react in a way similar to the phenols of other elastic stains (Fullmer, 1965). The fact that alcohol soluble aniline blue (spirit blue) also contains aromatic amine groups (Gurr, 1962) is probably therefore of significance in the staining reactions of the spirit blue technique.

While the above may be of value in attempting to explain the ability of spirit blue to act as a selective stain for vertebrate elastic fibers it does not explain the requirement of the invertebrate fibers for oxidation before a positive reaction can be obtained with spirit blue or aldehyde fuchsin. The oxytalan fibers of the human periodontal membrane will similarly give positive staining with aldehyde fuchsin only after oxidation (Fullmer and Lillie, 1958), and there is strong evidence that these staining properties of the oxytalan fibers are due to conjugated mucopolysaccharide (Fullmer, 1965).

As discussed above some of the invertebrate connective tissue elements which stain strongly by the spirit blue method may have no physical elasticity or chemical affinity with elastic proteins and may stain only by virtue of associated glycosaminoglycans. Even amongst those fibers for which there is some indirect evidence of physical elasticity from anatomical considerations there appear to be several morphological types. Thus the annelids and molluscs have fibers composed of bundles of fine beaded filaments while in the coelenterates and arthropods the fibers are composed of compound fibrils each of which consists of aggregates of fine filaments apparently crosslinked. These structural differences may reflect differences in chemical composition and mechanical properties.

The mechanical properties of the vertebrate elastin precursor fiber do not appear to have been studied in detail. Amino acid analysis of purified samples have shown that, in contrast to the mature "amorphous" elastin, the fibrillar component is a glycoprotein rich in polar amino acids (Ross and Bornstein, 1969, 1970). Therefore the molecular organization and elastic properties of the precursor filaments (if indeed they are elastic) cannot be due to the type of two phase model involving interlinked hydrophobic globules in an aqueous phase, proposed by Partridge (1968, 1970) or the "liquid drop elastomer" of Weis-Fogh and Andersen (1970). An amino acid analysis of purified spirit blue staining fibers would be

of great interest and relevance although the presence of numerous polar groups need not deny rubber-like properties, as Bailey and Weis-Fogh (1961) and Weis-Fogh (1965) have shown for the protein resilin from arthropod cuticle. In ultra-thin sections vertebrate elastin usually appears amorphous (Cox and Little, 1961; Ross and Bornstein, 1969). Partridge (1968) has recently presented micrographs which reveal a network of globular particles in a three dimensional array and considers that the beaded fibrillar apparance of some negatively stained elastin preparations (Gotte, Meneghelli and Castellani, 1965; Gotte, Mammi and Pezzin, 1968) has relevance only to a super stretched condition. Weis-Fogh and Andersen (1970; page 721) however point out that "one advantage of a liquid drop elastomer is that one can imagine the existence of elastic fibrils of much smaller diameter than is possible for rubberlike elastomers, because a single linear row of interlinked globular molecules would show an elastic behavior which is similar to that of a larger aggregate of globules." Further mechanical, ultrastructural and chemical studies of the invertebrate fibers described above are clearly required before many of the questions raised above can be answered.

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Summary

1. A microscopical survey of some forty species from twelve invertebrate phyla confirmed the presence of collagen in all groups.

2. A second group of connective tissue fibers, distinguishable from collagen by permanganate/spirit blue staining and which includes invertebrate elastic fibers was found in all groups except the anthozoan coelenterates and the turbellarians.

3. Spirit blue positive fibers were variously found in the dermis, around nerves, amongst muscles, in blood vessels and epithelial basement membranes and traversing the mesoglea of several coelenterate types. It is probable that further work will confirm the physical elasticity of many of these fibers.

4. Anatomically the "spirit blue fibers" are oriented to antagonize muscles in the medusae, accommodate fluid pressures in vascular systems and oppose tissue deformation in many soft-bodied animals.

5. Specifically, in the latter category, radially oriented dermal "spirit blue fibers" oppose the radial distension of the body wall during the simultaneous circular and longitudinal muscle contraction which occurs in the passage of direct peristaltic waves in the burrowing holothurian *Leptosynapta tenuis*.

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